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(54) OXIDASE ORIGINATED FROM BACTERIA

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain an oxidase of an extracellular product of a bacterium belonging to the genus of Bacillus, having an oxidizing action of polyphenols, and useful for the oxidizing treatment of a colored substance and the oxidizing treatment, cleaning, etc., of a substance containing polyphenols.

SOLUTION: This oxidase is obtained by culturing Bacillus licheniformis SD3003 (FERM P-15383), etc., and is a polyphenoloxidase having the following properties: (1) oxidizes polyphenols and has a substrate specificity of ferulic acid, syringaldazine, o-pheylenediamine, etc.; (2) has an optimum reaction pH of approximately 7; (3) has an optimum reaction temperature of at 60-80°C; and (4) has a molecular weight of approximately 51,000 (by a GFC analysis). The oxidase is useful for various treatments such as breaching treatment and cleaning treatment of a paper, a pulp and a fiber, the treatment of microorganisms or viruses and the like.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] The technical problem which this invention tends to solve offers the polyphenol oxidase which bacteria produce, its production microorganism, and an application, and offers the new enzyme source of supply for using this enzyme for oxidation treatment of the coloring matter, oxidation treatment of a polyphenol inclusion, and washing.

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EFFECT OF THE INVENTION

[Effect of the Invention] The polyphenol oxidase which bacteria produce by this invention was offered, enzyme-oxidation was attained by using this, and it was shown that it can contribute to oxidation treatment of the polyphenol matter or the coloring matter and use of polyphenol oxidases, such as washing and bleaching, as explained to the detail above. Moreover, the polyphenol oxidase of this invention can be efficiently manufactured by the manufacture approach of the polyphenol oxidase of this invention.
[0044] Moreover, Bacillus licheniformis of this invention SD3003 is effective in manufacture of the polyphenol oxidase of this invention. Moreover, approaches, such as processing of processing of processing of the effective coloring matter, paper, pulp, or fiber, bleaching processing, washing processing, a microorganism, or a virus, are offered by using the polyphenol oxidase of this invention.

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PRIOR ART

[Description of the Prior Art] Conventionally, the polyphenol oxidase and the laccase are known as an enzyme with the polyphenol oxidation. And it was [that mold, such as Basidiomycetes and fungi imperfecti, is only known and] as a production bacillus of these enzymes. On the other hand, researches and developments are actively done about the usage of polyphenol oxidase, WO 94-29510 etc. has a report about the delignification in paper and the pulp field, and the use for bleaching by the washing actuation by wash etc. is indicated by WO 91-05839, EP91610032 and DE4008894, JP,64-60693,A, etc. However, since the production bacilli of the conventional polyphenol oxidase or a laccase are mold, such as Basidiomycetes and fungi imperfecti, generally, mass culture is difficult and its growth rate at the time of culture is not high, either. Moreover, in order to raise the productivity of the purpose enzyme, when performing acquisition and genetic manipulation of a variant, there is much what requires a great effort compared with bacteria because of the complexity of gene structures, such as a complicated life cycle which these funguses have, and existence of the intron. Since it was such, it was cheaply difficult stability and to mass-produce a polyphenol oxidase by the fungus, and in order to present the use on industry, a polyphenol oxidase of the bacteria origin was desired.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the polyphenol oxidase which bacteria produce, its production microorganism, and an application. Furthermore, the new enzyme source of supply for oxidation treatment of the coloring matter by the polyphenol oxidase, oxidation treatment of a polyphenol inclusion, and washing is offered in detail.
[0002]
[Description of the Prior Art] Conventionally, the polyphenol oxidase and the laccase are known as an enzyme with the polyphenol oxidation. And it was [that mold, such as Basidiomycetes and fungi imperfecti, is only known and] as a production bacillus of these enzymes. On the other hand, researches and developments are actively done about the usage of polyphenol oxidase, WO 94-29510 etc. has a report about the delignification in paper and the pulp field, and the use for bleaching by the washing actuation by wash etc. is indicated by WO 91-05839, EP91610032 and DE4008894, JP,64-60693,A, etc. However, since the production bacilli of the conventional polyphenol oxidase or a laccase are mold, such as Basidiomycetes and fungi imperfecti, generally, mass culture is difficult and its growth rate at the time of culture is not high, either. Moreover, in order to raise the productivity of the purpose enzyme, when performing acquisition and genetic manipulation of a variant, there is much what requires a great effort compared with bacteria because of the complexity of gene structures, such as a complicated life cycle which these funguses have, and existence of the intron. Since it was such, it was cheaply difficult stability and to mass-produce a polyphenol oxidase by the fungus, and in order to present the use on industry, a polyphenol oxidase of the bacteria origin was desired.
[0003]
[Problem(s) to be Solved by the Invention] The technical problem which this invention tends to solve offers the polyphenol oxidase which bacteria produce, its production microorganism, and an application, and offers the new enzyme source of supply for using this enzyme for oxidation treatment of the coloring matter, oxidation treatment of a polyphenol inclusion, and washing.
[0004]
[Means for Solving the Problem] The polyphenol oxidase and the laccase are known as an enzyme with the polyphenol oxidation. And it was [that mold, such as Basidiomycetes and fungi imperfecti, is only known and] as a production bacillus of these enzymes. Then, this invention persons looked for the fungus body exogenous product which carries out the catalyst of the oxidization of the polyphenol matter wholeheartedly in extensive bacteria. Although the retrieval was very difficult, it finds out that the strain belonging to a bacillus (Bacillus) group produces the target enzyme out of a fungus body at last, and it came to complete this invention.
[0005] That is, this invention offers the following.
1) The polyphenol oxidase of the bacteria origin.
2) The polyphenol oxidase of said one publication which has the following property.
(1) Oxidize operation polyphenol.

(2) It has the optimum reaction pH in the optimum reaction pHpH7 neighborhood.
(3) It has optimum reaction temperature in optimum reaction temperature of 60-80 degrees C.
(4) The molecular weight measured by molecular weight GFC analysis is about 51,000.
[0006] 3) The polyphenol oxidase of said 1 or 2 publications of the bacillus (Bacillus) group bacteria origin.
4) Said polyphenol oxidase of three publication whose bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacillus licheniformis) or Bacillus natto (Bacillus natto).
5) The polyphenol oxidase of said 1 or 2 publications which can be obtained from Bacillus licheniformis (Bacillus licheniformis) SD 3003 (trust number FERM P-15383).
[0007] 6) The art of the phenolic compound and alkoxyl group content aromatic series which are characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5, a halogenation phenolic compound, or an aromatic amine compound.
7) The art of the coloring matter characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5.
8) The art of the coloring wastewater characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5.
9) The art of the microorganism or virus characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5.
10) The art of the paper characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5, pulp, or fiber.
11) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by using with a cleaning agent, a detergent, or a surfactant.
[0008] 12) The detergent constituent characterized by including the polyphenol oxidase of a publication in said any 1 term of 1-5.
13) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by using with the matter which has a peroxidase operation.
14) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by being independent, or combining two or more air, oxygen, ozone, a hydrogen peroxide, hydrogen-peroxide precursors, peroxy-acid precursors, or peroxy acids, and using them as an oxidizer.
15) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by using with an oxidase and its substrate.
[0009] 16) The manufacture approach of a polyphenol oxidase given in said any 1 term of 1-5 characterized by cultivating bacillus (Bacillus) group bacteria.
17) The manufacture approach of the polyphenol oxidase said 16 publication that bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacilluslicheniformis) or Bacillus natto (Bacillus natto).
18) The manufacture approach of the polyphenol oxidase said 16 publication that bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacilluslicheniformis) SD 3003 (trust number FERM P-15383) or its variant.
[0010] 19) The bacillus (Bacillus) group bacteria which produce the polyphenol oxidase of a publication in said any 1 term of 1-5.
20) Bacillus licheniformis SD 3003 (trust number FERM P-15383) (Bacillus licheniformis).
[0011] This invention is explained below at a detail.
(Production bacillus) The strain belonging to Bacillus used in order to obtain the polyphenol oxidase of this invention that what is necessary is just to have this polyphenol oxidase productivity Although there is especially no limit except it, for example Bacillus ARUKARO philus (Bacillus alcalophilus), Bacillus amyloliquefaciens (Bacillus amyloliquefaciens), Bacillus brevis (Bacillus brevis), a bacillus fur mass (Bacillus firmus), Bacillus licheniformis (Bacillus licheniformis), Bacillus natto (Bacillus natto), Bacillus pumilus (Bacillus pumilus), Although bacillus SUFAERIKASU (Bacillus sphaericus), bacillus Subtilis (Bacillus subtilis), etc. are mentioned Preferably Bacillus licheniformis (Bacilluslicheniformis), Bacillus natto (Bacillus natto), It is Bacillus licheniformis especially preferably. SD3003 (Bacillus licheniformis SD3003) (it ****s to National Institute of Bioscience and Human-Technology, Agency of

Industrial Science and Technology as FERM P-15383) is used. the typical strain in this invention -- gestalt observation -- physiological -- description -- results, such as a trial and measurement, are shown below.

[0012]

---------- An item A result ---------- A gestalt A ** ** bacillus Gram's stain nature + A spore + Form Ellipse form Location Neutrality - ****** sporangium Un-bulging. Maneuverability + The attitude against oxygen a denominator -- anaerobiosis A catalase + The growth under an aversion + A V-P reaction + pH of V-P broth 5.2 Generation of the acid from a glucose + Generation of the gas from a glucose - Liquefaction of gelatin + Decomposition [of starch] + Use of citrate + Use of propionate + An egg yolk reaction - Reduction of a nitrate + pH6.8 Growth (nutrient broth) + pH5.7 Growth + The growth under 5%NaCl existence + Growth under 7%NaCl existence + Growth at 10 degrees C - Growth at 30 degrees C + Growth at 55 degree C +65 degrees C growth - Inside DNA of a fungus body GC content (mol %) * 46 ---------- It was based on the * HPLC method.

[0013] It refers to reference ("Bergey's Manual of Systematic Bacteriology" Vol.2 (1986) Williams & Wilkins and "The Genus Bacillus" (1973) U.S.Department of Agriculture), and a bacteria stock is [these results and] Bacillus licheniformis. It was named SD3003 (Bacillus licheniformis SD3003).

[0014] (Preparation of an enzyme) The polyphenol oxidase of this invention cultivates the strain belonging to aforementioned Bacillus, and its variant, and is obtained, and also it can be prepared using a genetic manipulation bacillus. DNA which carries out the code of this polyphenol oxidase with namely, the suitable promotor who has an enzyme manifestation function in a host organism and an operator, and Terminator DNA The host cell by which the transformation was carried out using the expression vector inserted in the DNA vector which has the origin of replication for reproducing a vector in the host organism, DNA which carries out the code of this polyphenol oxidase with or the suitable promotor who has an enzyme manifestation function in a host organism and an operator, and Terminator DNA It cultivates on the basis of the conditions on which a polyphenol oxidase can discover the host cell by which the transformation was carried out by carrying out integration to a host cell DNA, and is produced also by the approach of collecting polyphenol oxidases from a culture medium further.

[0015] For acquisition of the DNA fragment which carries out the code of the polyphenol oxidase of this invention For example, cDNA or the genomic library from strain of this invention is made into the source of separation. [whether the target DNA fragment is specified by using as a probe the oligonucleotide compounded based on the amino acid sequence of the polyphenol oxidase of this invention, or the amino acid sequence of a known polyphenol oxidase, and] Or it can carry out with the conventional method of choosing the clone which discovers enzyme activity or choosing the antibody to this polyphenol oxidase, and the clone which produces the protein which reacts.

[0016] The culture for obtaining the polyphenol oxidase of this invention has an usable nutrition culture medium including the synthetic medium usually used, the source of organic carbon, and the source of organic nitrogen. Moreover, it is desirable to add by the concentration of 0.01mM(s) to 1mM preferably 10 mMs from 0.001mM(s) by making Cu2+ ion into a metal salt. Moreover, it is desirable to add by the concentration of 0.01mM(s) to 10mM(s) preferably 100 mMs from 0.001mM(s) by making Mn2+ ion into a metal salt. 20-60 degrees C of culture temperature are 30-55 degrees C preferably. Moreover, suitable culture time amount is 150 hours from 40 hours preferably from 20 hours for 200 hours. The secreted polyphenol oxidase is recoverable by the well-known approach out of a culture medium. Centrifugal separation or filtration, and membrane separation separate a cell into this recovery procedure from a culture medium, for example, a series of procedures of performing the chromatography by ion exchange chromatography etc. are included in it. Moreover, the membrane concentration using ultrafiltration membrane is also effective. Moreover, separation concentration is possible also by the salting-out using an ammonium sulfate etc.

[0017] (Property of an enzyme) Bacillus licheniformis which is the example of representation of the polyphenol oxidase of this invention Although the enzyme of the SD3003 origin can be oxidized in [large / pH] 5-9, preferably, it is the pH7 neighborhood more preferably (drawing 1), and has pH 6-8

and the features of carrying out the catalyst of the oxidation reaction in a neutral pH region. Moreover, optimum temperature is 60-80 degrees C (drawing 2), and the activity after performing heat-treatment for 30 minutes with various constant temperature in pH7 shows about 100% of residual activity in 70 degrees C further (drawing 3). Furthermore, the residual activity after performing 30 degrees C and processing for 30 minutes in the buffer of various pH shows the stability in wide range pH (drawing 4). These results are wide range pH regions of the acescence to alkalescence, and guarantee oxidation reaction in various solutions of inside low temperature. Moreover, the molecular weight by GFC analysis is about 51,000.

[0018] Moreover, with the enzyme which has the optimum reaction pH, it combines and the polyphenol oxidase of this invention can also be used for the conventional acidity side. That is, it becomes possible to perform a polyphenol oxidase reaction in wide range weak alkaline pH region from acidity by using for the acidity side known conventionally combining the polyphenol oxidase which has the optimum reaction pH, and the polyphenol oxidase of this invention. the mixing ratio of the active mass of the polyphenol oxidase which has the optimum reaction pH in an acidity side when mixing and using an enzyme for such the purpose, and the active mass of the polyphenol oxidase of this invention -- a rate -- desirable -- 1:10-10:1 -- it is 1:3-3:1 more preferably. Thus, also in order to attain a polyphenol oxidase reaction in wide range pH region, the polyphenol oxidase of this invention is useful.

[0019] (Activity measurement method) In this invention, the activity measurement of polyphenol oxidization activity reacted in the water solution which contains 20 ppm syringaldazine (syringaldazine) and the Bis-Tris-HCl buffer solution (pH7.0) (Bis-Tris comes to hand from a dotite reagent) of 100mM (s) in 20 degrees C, and was performed by measuring the absorbance of 525nm. And the active mass which oxidizes the syringaldazine of 1nmol in 1 minute was defined as 1munit (it omits Following mU). Moreover, when processing a polyphenol inclusion using the polyphenol oxidase of this invention, it was used by ten to 500 mU/ml activity concentration.

[0020] (Application) As an application of a polyphenol oxidase, application to bleaching is possible, for example. Use to bleaching of polyphenol oxidase is indicated by WO 91-05839, DE4008894, JP,64-60693,A, etc. The polyphenol oxidase of this invention is useful as what opens the application of the enzyme of the bacteria origin to such washing and the bleaching field.

[0021] Oxidation bleaching by the hydrogen peroxide is widely used in washing and wash now. However, low temperature 60 degrees C or less of a hydrogen peroxide is not enough as the bleaching force. Although the peroxy-acid precursor is used with the hydrogen peroxide in order to improve this, the bleaching force in low temperature 40 degrees C or less is not enough, and the high bleaching system of effectiveness is called for more. Therefore, the various enzyme-bleaching promotion approaches are proposed conventionally. Being able to promote oxidation bleaching by making the polyphenol oxidase indicated here coexist with one or the plurality of the matter which has a peroxidase operation of a peroxidase, a lignin peroxidase, a manganese peroxidase, etc., the usefulness of this invention is in **.

[0022] The hydrogen peroxide used widely because of oxidation bleaching is an expensive oxidizer, and the hydrogen-peroxide precursor often used for the cleaning agent and a peroxy-acid precursor, and a peroxy acid are still more expensive oxidizers. Moreover, it is possible to generate a hydrogen peroxide in enzyme by using an oxidase and its substrate, and such a hydrogen-peroxide generating system can also be considered to be an expensive oxidizer. Oxidation bleaching can be promoted by in addition to the polyphenol oxidase indicated here, being independent, or combining two or more the air which is the oxidizer conventionally used for oxidation bleaching, oxygen, ozone, a hydrogen peroxide, hydrogen-peroxide precursors, peroxy-acid precursors, or peroxy acids, and using them. Therefore, the usefulness of this invention which can attain oxidation bleaching, using an oxidizer effectively is clear.

[0023] Among oxidizers, a hydrogen-peroxide precursor dissolves in water and generates par hydroxyl ion. In such matter, it is the perborate of one hydrate or four hydrates, par carbonate, a fault borax, a fault sodium pyrophosphate, a perbenzoic acid, and urea-H2 O2. A reactant and melamine-H2 O2 There are a reactant, a citric-acid fault hydrate, etc. and they are perborate and par carbonate especially preferably. Furthermore, the hydrogen-peroxide generating system by the oxidase and its substrate can

also be used as a hydrogen-peroxide precursor. The example of such an oxidase has glucose oxidase, alcohol oxidase, a glycerol oxidase, amine oxidase, amino acid oxidase, D-amino-acid oxidase, aryl alcohol oxidase, aldehyde oxidase, galactose oxidase, a sorbose oxidase, urate oxidase, xanthine oxidase, cholesterol oxidase, etc., and are glucose oxidase and alcohol oxidase especially preferably.
[0024] Moreover, the organic compound with which a peroxy-acid precursor has a reactant acyl group or carboxylate, They are a carboxylic anhydride and acetate. To such matter TAED (tetraacetylethylenediamine), TAMD (tetraacetylmethylenediamine), TAGU (tetraacetylglycoluril), DADHT (diacetyldioxohexahydrotriazine), SNOBS (sodium nonanoyloxybenzene sulfonate), ISONOBS (sodium isononanoyloxybenzene sulfonate), There are a succinic-acid anhydride, a benzoic anhydride, a phthalic-acid anhydride, PAG (glucose pentaacetate), and xylose tetra-acetate, and they are TAED and SNOBS especially preferably. Furthermore, peroxy acids are DPDDA (diperoxydodecanedioic acid), diperoxyisophthalic acid, magnesium monoperoxyphthalate hexahydrate, and NAPAA (nonylamidoperoxyadipic acid).
[0025] The polyphenol oxidase of this invention can be used with various cleaning agents, a detergent, or a surfactant. Thereby, the cleaning agent or detergent constituent which blended the polyphenol oxidase of this invention is offered. The cleaning agent or detergent constituent which consists of at least one or more sorts of combination components chosen from the group which such an example of representation of a cleaning agent or a detergent constituent becomes from a cleaning agent or 10 - 50% of the weight per detergent constituent weight of a surface active agent, 0 - 50% of the weight of a builder, 1 - 50% of the weight of alkali chemicals or an inorganic electrolyte, 0.1 - 10% of the weight of an anti-redeposition agent, an enzyme, a bleaching agent, fluorescent dye, a caking inhibitor, and an antioxidant is mentioned.
[0026] As a surfactant, soap, for example, a straight chain, branching alkyl, or an alkenyl sulfate, It has the alkyl group or alkenyl radical of an amidosulfuric acid salt, a straight chain, or branched chain. Independent or the alkyl which two or more components added or an aliphatic series sulfation object like an alkenyl ethereal sulfate salt of ethyleneoxide, propylene oxide, and the butylene oxide, An alkyl sulfonate, an amidosulfonic acid salt, dialkyl sulfo succinate, An aliphatic series sulfonate like each sulfonate of an alpha olefin, a vinylidene mold olefin, and an internal olefin, An aromatic series sulfonate like the alkylbenzene sulfonates of a straight chain or branched chain, It has the alkyl group or alkenyl radical of a straight chain or branched chain. Ethyleneoxide, Independent, the alkyl which two or more components added or alkenyl ether carboxylate of propylene oxide and the butylene oxide, or an amide, alpha-sulfo fatty-acid salt or ester, an amino acid mold surfactant, alkyl, or alkenyl alkyl acid phosphate, Alkyl or the phosphoric ester system surfactant like alkenyl phosphate, It has the alkyl group or alkenyl radical of a sulfonic acid type amphoteric surface active agent, a betaine mold amphoteric surface active agent, a straight chain, or branched chain. Independent, the alkyl which two or more components added or the alkenyl ether of ethyleneoxide, propylene oxide, and the butylene oxide, or alcohol, It has the alkyl group or alkenyl radical of a straight chain or branched chain. Ethyleneoxide, Independent or polyoxyethylene alkyl phenyl ether which two or more components added of propylene oxide and the butylene oxide, A higher-fatty-acid alkanol amide or its alkylene oxide addition product, Sucrose fatty acid ester, fatty-acid glycerol monoester, alkyl, or alkenyl amine oxide, If it is the surfactant usually blended as a detergent constituent, such as a tetra-alkyl-ammonium-salt mold cationic surface active agent, all are usable, and it is desirable that they are sodium ion or potassium ion as a counter ion in the case of an anionic detergent. These surfactants are used as independent or two or more sorts of mixture.
[0027] As a builder and alkali chemicals, or an inorganic electrolyte, orthophosphate, A pyrophosphate, Tripoli acid chloride, a metaphosphate, a hexametaphosphoric acid salt, Phosphate, ethane -1, 1-diphosphonic acid, and its derivatives, such as a phytic acid salt, Ethane hydroxy - 1, 1, 2-triphosphonic acid, ethane -1, 2-dicarboxy - 1, 2-diphosphonic acid, Phosphonate, such as methane hydroxy phosphonic acid, 2-phosphono butane -1, 2-dicarboxylic acid, 1-phosphono butane - Phosphono carboxylate, such as 2, 3, 4-tricarboxylic acid, and alpha-methyl phosphono succinic acid, Amino acid salts, such as an aspartic acid and glutamic acid, a nitrilotriacetic acid salt, Amino poly acetate, such as

an ethylenediaminetetraacetic acid salt and a diethylenetriamine pentaacetic acid salt, Polyacrylic acid, the Pori itaconic acid, a polymer lane acid, a maleic-anhydride copolymer, Polyelectrolytes, such as a carboxymethyl-cellulose salt, a polyethylene glycol, Non-dissociating giant molecules, such as polyvinyl alcohol, diglycolic acid, an oxydi succinic acid, Carboxymethyl malic acid, a gluconic acid, a citric acid, a lactic acid, a tartaric acid, Carboxymethyl ghosts, such as cane sugar and a lactose, the carboxymethyl ghost of pentaerythritol, The carboxymethyl ghost of a gluconic acid, benzene polycarboxylic acid, oxalic acid, Aluminosilicates, such as organic-acid salts, such as a malic acid, an oxydi succinic acid, and a gluconic acid, and a zeolite, Mineral salt, such as a carbonate, a sesquicarbonate, a sulfate, and a metasilicate, can be used as an alkali-metal salt. Moreover, inorganic compounds, such as organic substances, such as starch and a urea, and a sodium chloride, and a bentonite, can be used. Furthermore, triethanolamine, diethanolamine, monoethanolamine, tri-isopropanolamine, etc. can be used as organic alkali chemicals.

[0028] Although the detergent constituent of this invention contains the polyphenol oxidase of a surfactant and this invention etc. as a constituent like the above-mentioned, it can include the enzyme of others, such as anti-redeposition agents, such as bleaching agents, such as an amphoteric surface active agent, for example, perborate, and par carbonate, coloring matter, a builder, for example, a polyethylene glycol, polyvinyl alcohol, a polyvinyl pyrrolidone, and a carboxymethyl cellulose, a caking inhibitor, an antioxidant, for example, other oxidases and peroxidases, a protease, lipase, an amylase, and a cellulase, if needed if needed [the].

[0029] Although you may carry out with what kind of approach for blending an enzyme with the detergent constituent of this invention, blending by the shape of impalpable powder is not desirable for reasons of sanitation [of the detergent user by the raising dust at the time of detergent handling, or the operator in detergent industry / insurance], and it is desirable to carry out size enlargement to a solution condition or the configuration where dusting characteristics were pressed down beforehand. This size enlargement may be based on any of the approach of the Malmo grain usually used well, extrusion granulation, fluidized bed granulation, centrifugal fluidized bed granulation, or others, and especially the configuration of the enzyme blended with the detergent constituent of this invention is not limited to that by which size enlargement was carried out by these approaches.

[0030] In a part of pulp process, inoculate the strain of this invention for the purpose of delignification and bleaching, and the polyphenol oxidase of this invention is made to produce, or the enzyme preparation of direct this invention is added, and the biotechnology PAL ping and biotechnology bleaching which are made to act on a chip, crushing pulp, etc. are mentioned to the useful application field.

[0031] As a natural product which has polyphenol in a part for the structured division, plant pigment and lignins, such as a flavonoid system, a xanthone system, and a melanin system, are known, and a polyphenol oxidase has the oxidation to these natural products. Moreover, AOX(s) from which toxicity has been a problem, such as dichlorophenol and trichlorophenol, are also made as for a polyphenol oxidase to a reaction substrate. So, also in the waste water treatment which contains these natural products and non-natural products, for example, the polyphenol oxidase of this invention is useful.

[0032] Moreover, the biosensor using the polyphenol oxidase of this invention can be used in order to act as the monitor of the aromatic compound in various water solutions which have pH of an acescence - weak alkaline region, and an organic solvent reflecting the property of this enzyme, and it is useful. Moreover, it is also possible to perform sterilization and inactivation of a microorganism or a virus efficiently in weak acidic - weak alkaline pH region using the reactivity of the phenoxyl radical generated by the polyphenol oxidase of this invention. That is, it is possible to give disinfectant [more powerful] by the phenoxyl radical generated in enzyme in addition to disinfectant [of the substrate of a polyphenol oxidase itself]. And when a sterilization processing object is contacted or taken in by the after body, or when being emitted into an environment, since the substrate of a polyphenol oxidase has changed to the matter with which toxicity was mitigated by oxidation, when required, it can attain the both sides of disinfectant and subsequent safety, and usefulness is high [the substrate].

[0033] Moreover, also in the polymer composition using the phenoxyl radical generated by the

polyphenol oxidase, or quinones, the polyphenol oxidase of this invention is useful. Furthermore, since an autoxidation reaction and oxidation reaction of enzymatic catalyst-polyphenol can be advanced to coincidence when oxidizing the matter group containing easy-oxidizable polyphenol, such as catechols, use of the polyphenol oxidase of this invention is very effective because of efficient oxidation.
[0034]
[Example] A typical example is shown about this invention below, and it explains to it still more concretely. However, these are mere instantiation and this inventions are not these things restricted for seeing.
[0035] Example 1 : The flask of culture and rough purification, and 500ml ** of concentration is used for a culture apparatus. 0.134%Na2 HPO4 and 12H2 O, and 0.03%KH2 PO4, 1% maltose, 1% peptone, 0.1% yeast extract, 0.05%MgSO4 and 7H2 O, 0.1mM(s) CuSO4, 1mM MnCl2, 2mM CaCl2 It is 20% Na2 CO3 to the included 100ml culture medium. To what set pH to 7.8 in addition Bacillus licheniformis SD3003 (trust number FERM P-15383) was inoculated, culture temperature was lowered to 35 degrees C after 50 degrees C and the shaking culture of 16 hours, and culture for three more days was performed. The culture broth disinfected by 4-degree C centrifugal separation was obtained after culture. In order to refine and condense this further, ammonium sulfate fractionation was effective and was able to collect a great portion of polyphenol oxidase activity as precipitation in saturated-ammonium-sulfate concentration 20 to 60%. The obtained ammonium sulfate precipitation is 10mM(s). It dialyzed to the Bis-Tris-HCl buffer solution (pH7.0), in order to refine and condense further, ultrafiltration membrane was used, and the rough purification retentate solution (800 mU/ml) was obtained to the fractionation range of molecular weight 10,000-100,000.
[0036] Example 2: The substrate specificity of polyphenol compound oxidation reaction was investigated using the rough purification retentate solution of substrate specificity example 1 publication. In the room temperature (20 degrees C), it carried out by measuring the difference of the substrate of 0.05mM, and the enzyme addition in a 100 mMBis-Tris-HCl buffer solution (pH7.0) and an additive-free oxygen consumption coefficient. The result was shown in Table 1.
[0037]
[Table 1]

基質	酸化反応
シリンガルダジン	+
4－アニシジン	+
o-フェニレンジアミン	+
フェルラ酸	+

[0038] Example 3: The molecular weight determination of molecular weight was performed using GFC (gel filtration chromatography). When analysis, and the aliquot and activity measurement of a rough purification retentate solution of example 1 publication were performed, elution of the polyphenol oxidase activity peak was carried out to the range of molecular weight 46,000-56,000 by 1.34%Na2 HPO4 and 12H2 O, 0.3%KH2 PO4, and HPLC using the GFC column (Shodex PROTEINKW-802.5, 2 ream) and UV detector (280nm) which equilibrated by rate-of-flow 1.0 ml/min by NaCl 1%. In addition, MW-Marker (HPLC) of Oriental Industry was used for molecular weight marker protein.
[0039] Culture with the 4:5l. cultivation tank of examples and concentration, 0.134% Na2 HPOof rough purification4 and 12H2 O, 0.03%KH2 PO4, 1% maltose, 1% peptone, 0.1% yeast extract, 0.05%MgSO4 and 7H2O, 0.1mM CuSO4, 1mM MnCl2 and 2mM CaCl2 from -- to the 5l. cultivation tank containing what added NaOH to the 3l. culture medium which changes 10%, and set pH to 7.8 Bacillus licheniformis SD3003 (trust number FERM P-15383) was inoculated, culture temperature was lowered to 35 degrees C after 50 degrees C and the shaking culture of 16 hours, and culture for three more days was performed. The culture broth disinfected by 4-degree C centrifugal separation was obtained after culture.
[0040] Next, a part of this culture broth was condensed as a with a molecular weight of 10,000 or more fraction by the mini tongue ultrafiltration system (Millipore Corp. make) which uses a mini tongue filter

packet (CAT.NO.: PTGC0MP04, Millipore Corp. make). Furthermore, this concentration liquid is 200ppmNH(s)4 HCO3. It received, freeze drying was presented after dialysis, and the rough purification object was obtained as a freeze-drying article. The polyphenol oxidase activity of a freeze-drying article was 500 mU(s)/mg.

[0041] straight chain alkyl benzene sodium sulfonate (LAS) of 25 % of the weight of processings of the contamination cloth by the Example 5:enzyme content detergent, 5% of the weight of the polyoxyethylene lauryl ether, 15% of the weight of sodium tripolyphosphate, 6% of the weight of a sodium silicate, 1% of the weight of carboxymethylcellulose sodium, and 48% of the weight of Na2 SO4 from -- what added 0.1g of freeze-drying articles of example 4 publication in 10g of becoming index detergents was used as the enzyme combination detergent, and what has not added the freeze-drying article be used as enzyme the non-blending detergent. Moreover, the contamination cloth was prepared by adding 100 ppm Evans Blue (Wako Pure Chem Industry) 0.2ml in the center of cotton white cloth (5cmx5cm).

[0042] Next, after adding one contamination cloth and 10ml of water to 500ml ** beaker, washing processing was performed by 10mg adding and shaking an enzyme combination detergent, or enzyme a non-blending detergent for 12 minutes further. When the contamination cloth after processing was rinsed and air-dried, Y, y, and x values were measured with the color color difference meter (CR-200 and MINOLTA make) and Z value was further computed by the formula [Z=(1-x-y) Y/y], the enzyme combination detergent showed the improvement in a whiteness degree of 1.5 points compared with the enzyme non-blending detergent.

[0043]

[Effect of the Invention] The polyphenol oxidase which bacteria produce by this invention was offered, enzyme-oxidation was attained by using this, and it was shown that it can contribute to oxidation treatment of the polyphenol matter or the coloring matter and use of polyphenol oxidases, such as washing and bleaching, as explained to the detail above. Moreover, the polyphenol oxidase of this invention can be efficiently manufactured by the manufacture approach of the polyphenol oxidase of this invention.

[0044] Moreover, Bacillus licheniformis of this invention SD3003 is effective in manufacture of the polyphenol oxidase of this invention. Moreover, approaches, such as processing of processing of processing of the effective coloring matter, paper, pulp, or fiber, bleaching processing, washing processing, a microorganism, or a virus, are offered by using the polyphenol oxidase of this invention.

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TECHNICAL FIELD

[Field of the Invention] This invention relates to the polyphenol oxidase which bacteria produce, its production microorganism, and an application. Furthermore, the new enzyme source of supply for oxidation treatment of the coloring matter by the polyphenol oxidase, oxidation treatment of a polyphenol inclusion, and washing is offered in detail.

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MEANS

[Means for Solving the Problem] The polyphenol oxidase and the laccase are known as an enzyme with the polyphenol oxidation. And it was [that mold, such as Basidiomycetes and fungi imperfecti, is only known and] as a production bacillus of these enzymes. Then, this invention persons looked for the fungus body exogenous product which carries out the catalyst of the oxidization of the polyphenol matter wholeheartedly in extensive bacteria. Although the retrieval was very difficult, it finds out that the strain belonging to a bacillus (Bacillus) group produces the target enzyme out of a fungus body at last, and it came to complete this invention.
[0005] That is, this invention offers the following.
1) The polyphenol oxidase of the bacteria origin.
2) The polyphenol oxidase of said one publication which has the following property.
(1) Oxidize operation polyphenol.
(2) It has the optimum reaction pH in the optimum reaction pHpH7 neighborhood.
(3) It has optimum reaction temperature in optimum reaction temperature of 60-80 degrees C.
(4) The molecular weight measured by molecular weight GFC analysis is about 51,000.
[0006] 3) The polyphenol oxidase of said 1 or 2 publications of the bacillus (Bacillus) group bacteria origin.
4) Said polyphenol oxidase of three publication whose bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacillus licheniformis) or Bacillus natto (Bacillus natto).
5) The polyphenol oxidase of said 1 or 2 publications which can be obtained from Bacillus licheniformis (Bacillus licheniformis) SD 3003 (trust number FERM P-15383).
[0007] 6) The art of the phenolic compound and alkoxyl group content aromatic series which are characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5, a halogenation phenolic compound, or an aromatic amine compound.
7) The art of the coloring matter characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5.
8) The art of the coloring wastewater characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5.
9) The art of the microorganism or virus characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5.
10) The art of the paper characterized by making the polyphenol oxidase of a publication act on said any 1 term of 1-5, pulp, or fiber.
11) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by using with a cleaning agent, a detergent, or a surfactant.
[0008] 12) The detergent constituent characterized by including the polyphenol oxidase of a publication in said any 1 term of 1-5.
13) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by using with the matter which has a peroxidase operation.
14) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by being

independent, or combining two or more air, oxygen, ozone, a hydrogen peroxide, hydrogen-peroxide precursors, peroxy-acid precursors, or peroxy acids, and using them as an oxidizer.
15) Operation of a polyphenol oxidase given in said any 1 term of 1-5 characterized by using with an oxidase and its substrate.
[0009] 16) The manufacture approach of a polyphenol oxidase given in said any 1 term of 1-5 characterized by cultivating bacillus (Bacillus) group bacteria.
17) The manufacture approach of the polyphenol oxidase said 16 publication that bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacilluslicheniformis) or Bacillus natto (Bacillus natto).
18) The manufacture approach of the polyphenol oxidase said 16 publication that bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacilluslicheniformis) SD 3003 (trust number FERM P-15383) or its variant.
[0010] 19) The bacillus (Bacillus) group bacteria which produce the polyphenol oxidase of a publication in said any 1 term of 1-5.
20) Bacillus licheniformis SD 3003 (trust number FERM P-15383) (Bacillus licheniformis).
[0011] This invention is explained below at a detail.
(Production bacillus) The strain belonging to Bacillus used in order to obtain the polyphenol oxidase of this invention that what is necessary is just to have this polyphenol oxidase productivity Although there is especially no limit except it, for example Bacillus ARUKARO philus (Bacillus alcalophilus), Bacillus amyloliquefaciens (Bacillus amyloliquefaciens), Bacillus brevis (Bacillus brevis), a bacillus fur mass (Bacillus firmus), Bacillus licheniformis (Bacillus licheniformis), Bacillus natto (Bacillus natto), Bacillus pumilus (Bacillus pumilus), Although bacillus SUFAERIKASU (Bacillus sphaericus), bacillus Subtilis (Bacillus subtilis), etc. are mentioned Preferably Bacillus licheniformis (Bacilluslicheniformis), Bacillus natto (Bacillus natto), It is Bacillus licheniformis especially preferably. SD3003 (Bacillus licheniformis SD3003) (it ****s to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology as FERM P-15383) is used. the typical strain in this invention -- gestalt observation -- physiological -- description -- results, such as a trial and measurement, are shown below.
[0012]
---------- An item A result ---------- A gestalt A ** ** bacillus Gram's stain nature + A spore + Form Ellipse form Location Neutrality - ****** sporangium Un-bulging. Maneuverability + The attitude against oxygen a denominator -- anaerobiosis A catalase + The growth under an aversion + A V-P reaction + pH of V-P broth 5.2 Generation of the acid from a glucose + Generation of the gas from a glucose - Liquefaction of gelatin + Decomposition [of starch] + Use of citrate + Use of propionate + An egg yolk reaction - Reduction of a nitrate + pH6.8 Growth (nutrient broth) + pH5.7 Growth + The growth under 5%NaCl existence + Growth under 7%NaCl existence + Growth at 10 degrees C - Growth at 30 degrees C + Growth at 55 degree C +65 degrees C growth - Inside DNA of a fungus body GC content (mol %) * 46 ---------- It was based on the * HPLC method.
[0013] It refers to reference ("Bergey's Manual of Systematic Bacteriology" Vol.2 (1986) Williams & Wilkins and "The Genus Bacillus" (1973) U.S.Department of Agriculture), and a bacteria stock is [these results and] Bacillus licheniformis. It was named SD3003 (Bacillus licheniformis SD3003).
[0014] (Preparation of an enzyme) The polyphenol oxidase of this invention cultivates the strain belonging to aforementioned Bacillus, and its variant, and is obtained, and also it can be prepared using a genetic manipulation bacillus. DNA which carries out the code of this polyphenol oxidase with namely, the suitable promotor who has an enzyme manifestation function in a host organism and an operator, and Terminator DNA The host cell by which the transformation was carried out using the expression vector inserted in the DNA vector which has the origin of replication for reproducing a vector in the host organism, DNA which carries out the code of this polyphenol oxidase with or the suitable promotor who has an enzyme manifestation function in a host organism and an operator, and Terminator DNA It cultivates on the basis of the conditions on which a polyphenol oxidase can discover the host cell by which the transformation was carried out by carrying out integration to a host cell DNA,

and is produced also by the approach of collecting polyphenol oxidases from a culture medium further.
[0015] For acquisition of the DNA fragment which carries out the code of the polyphenol oxidase of this invention For example, cDNA or the genomic library from strain of this invention is made into the source of separation. [whether the target DNA fragment is specified by using as a probe the oligonucleotide compounded based on the amino acid sequence of the polyphenol oxidase of this invention, or the amino acid sequence of a known polyphenol oxidase, and] Or it can carry out with the conventional method of choosing the clone which discovers enzyme activity or choosing the antibody to this polyphenol oxidase, and the clone which produces the protein which reacts.
[0016] The culture for obtaining the polyphenol oxidase of this invention has an usable nutrition culture medium including the synthetic medium usually used, the source of organic carbon, and the source of organic nitrogen. Moreover, it is desirable to add by the concentration of 0.01mM(s) to 1mM preferably 10 mMs from 0.001mM(s) by making Cu2+ ion into a metal salt. Moreover, it is desirable to add by the concentration of 0.01mM(s) to 10mM(s) preferably 100 mMs from 0.001mM(s) by making Mn2+ ion into a metal salt. 20-60 degrees C of culture temperature are 30-55 degrees C preferably. Moreover, suitable culture time amount is 150 hours from 40 hours preferably from 20 hours for 200 hours. The secreted polyphenol oxidase is recoverable by the well-known approach out of a culture medium. Centrifugal separation or filtration, and membrane separation separate a cell into this recovery procedure from a culture medium, for example, a series of procedures of performing the chromatography by ion exchange chromatography etc. are included in it. Moreover, the membrane concentration using ultrafiltration membrane is also effective. Moreover, separation concentration is possible also by the salting-out using an ammonium sulfate etc.
[0017] (Property of an enzyme) Bacillus licheniformis which is the example of representation of the polyphenol oxidase of this invention Although the enzyme of the SD3003 origin can be oxidized in [large / pH] 5-9, preferably, it is the pH7 neighborhood more preferably (drawing 1), and has pH 6-8 and the features of carrying out the catalyst of the oxidation reaction in a neutral pH region. Moreover, optimum temperature is 60-80 degrees C (drawing 2), and the activity after performing heat-treatment for 30 minutes with various constant temperature in pH7 shows about 100% of residual activity in 70 degrees C further (drawing 3). Furthermore, the residual activity after performing 30 degrees C and processing for 30 minutes in the buffer of various pH shows the stability in wide range pH (drawing 4). These results are wide range pH regions of the acescence to alkalescence, and guarantee oxidation reaction in various solutions of inside low temperature. Moreover, the molecular weight by GFC analysis is about 51,000.
[0018] Moreover, with the enzyme which has the optimum reaction pH, it combines and the polyphenol oxidase of this invention can also be used for the conventional acidity side. That is, it becomes possible to perform a polyphenol oxidase reaction in wide range weak alkaline pH region from acidity by using for the acidity side known conventionally combining the polyphenol oxidase which has the optimum reaction pH, and the polyphenol oxidase of this invention. the mixing ratio of the active mass of the polyphenol oxidase which has the optimum reaction pH in an acidity side when mixing and using an enzyme for such the purpose, and the active mass of the polyphenol oxidase of this invention -- a rate -- desirable -- 1:10-10:1 -- it is 1:3-3:1 more preferably. Thus, also in order to attain a polyphenol oxidase reaction in wide range pH region, the polyphenol oxidase of this invention is useful.
[0019] (Activity measurement method) In this invention, the activity measurement of polyphenol oxidization activity reacted in the water solution which contains 20 ppm syringaldazine (syringaldazine) and the Bis-Tris-HCl buffer solution (pH7.0) (Bis-Tris comes to hand from a dotite reagent) of 100mM (s) in 20 degrees C, and was performed by measuring the absorbance of 525nm. And the active mass which oxidizes the syringaldazine of 1nmol in 1 minute was defined as 1munit (it omits Following mU). Moreover, when processing a polyphenol inclusion using the polyphenol oxidase of this invention, it was used by ten to 500 mU/ml activity concentration.
[0020] (Application) As an application of a polyphenol oxidase, application to bleaching is possible, for example. Use to bleaching of polyphenol oxidase is indicated by WO 91-05839, DE4008894, JP,64-60693,A, etc. The polyphenol oxidase of this invention is useful as what opens the application of the

enzyme of the bacteria origin to such washing and the bleaching field.
[0021] Oxidation bleaching by the hydrogen peroxide is widely used in washing and wash now. However, low temperature 60 degrees C or less of a hydrogen peroxide is not enough as the bleaching force. Although the peroxy-acid precursor is used with the hydrogen peroxide in order to improve this, the bleaching force in low temperature 40 degrees C or less is not enough, and the high bleaching system of effectiveness is called for more. Therefore, the various enzyme-bleaching promotion approaches are proposed conventionally. Being able to promote oxidation bleaching by making the polyphenol oxidase indicated here coexist with one or the plurality of the matter which has a peroxidase operation of a peroxidase, a lignin peroxidase, a manganese peroxidase, etc., the usefulness of this invention is in **.
[0022] The hydrogen peroxide used widely because of oxidation bleaching is an expensive oxidizer, and the hydrogen-peroxide precursor often used for the cleaning agent and a peroxy-acid precursor, and a peroxy acid are still more expensive oxidizers. Moreover, it is possible to generate a hydrogen peroxide in enzyme by using an oxidase and its substrate, and such a hydrogen-peroxide generating system can also be considered to be an expensive oxidizer. Oxidation bleaching can be promoted by in addition to the polyphenol oxidase indicated here, being independent, or combining two or more the air which is the oxidizer conventionally used for oxidation bleaching, oxygen, ozone, a hydrogen peroxide, hydrogen-peroxide precursors, peroxy-acid precursors, or peroxy acids, and using them. Therefore, the usefulness of this invention which can attain oxidation bleaching, using an oxidizer effectively is clear.
[0023] Among oxidizers, a hydrogen-peroxide precursor dissolves in water and generates par hydroxyl ion. In such matter, it is the perborate of one hydrate or four hydrates, par carbonate, a fault borax, a fault sodium pyrophosphate, a perbenzoic acid, and urea-H2 O2. A reactant and melamine-H2 O2 There are a reactant, a citric-acid fault hydrate, etc. and they are perborate and par carbonate especially preferably. Furthermore, the hydrogen-peroxide generating system by the oxidase and its substrate can also be used as a hydrogen-peroxide precursor. The example of such an oxidase has glucose oxidase, alcohol oxidase, a glycerol oxidase, amine oxidase, amino acid oxidase, D-amino-acid oxidase, aryl alcohol oxidase, aldehyde oxidase, galactose oxidase, a sorbose oxidase, urate oxidase, xanthine oxidase, cholesterol oxidase, etc., and are glucose oxidase and alcohol oxidase especially preferably.
[0024] Moreover, the organic compound with which a peroxy-acid precursor has a reactant acyl group or carboxylate, They are a carboxylic anhydride and acetate. To such matter TAED (tetraacetylethylenediamine), TAMD (tetraacetylmethylenediamine), TAGU (tetraacetylglycoluril), DADHT (diacetyldioxohexahydrotriazine), SNOBS (sodium nonanoyloxybenzene sulfonate), ISONOBS (sodium isononanoyloxybenzene sulfonate), There are a succinic-acid anhydride, a benzoic anhydride, a phthalic-acid anhydride, PAG (glucose pentaacetate), and xylose tetra-acetate, and they are TAED and SNOBS especially preferably. Furthermore, peroxy acids are DPDDA (diperoxydodecanedioic acid), diperoxyisophthalic acid, magnesium monoperoxyphthalate hexahydrate, and NAPAA (nonylamidoperoxyadipic acid).
[0025] The polyphenol oxidase of this invention can be used with various cleaning agents, a detergent, or a surfactant. Thereby, the cleaning agent or detergent constituent which blended the polyphenol oxidase of this invention is offered. The cleaning agent or detergent constituent which consists of at least one or more sorts of combination components chosen from the group which such an example of representation of a cleaning agent or a detergent constituent becomes from a cleaning agent or 10 - 50% of the weight per detergent constituent weight of a surface active agent, 0 - 50% of the weight of a builder, 1 - 50% of the weight of alkali chemicals or an inorganic electrolyte, 0.1 - 10% of the weight of an anti-redeposition agent, an enzyme, a bleaching agent, fluorescent dye, a caking inhibitor, and an antioxidant is mentioned.
[0026] As a surfactant, soap, for example, a straight chain, branching alkyl, or an alkenyl sulfate, It has the alkyl group or alkenyl radical of an amidosulfuric acid salt, a straight chain, or branched chain. Independent or the alkyl which two or more components added or an aliphatic series sulfation object like an alkenyl ethereal sulfate salt of ethyleneoxide, propylene oxide, and the butylene oxide, An alkyl sulfonate, an amidosulfonic acid salt, dialkyl sulfo succinate, An aliphatic series sulfonate like each

sulfonate of an alpha olefin, a vinylidene mold olefin, and an internal olefin, An aromatic series sulfonate like the alkylbenzene sulfonates of a straight chain or branched chain, It has the alkyl group or alkenyl radical of a straight chain or branched chain. Ethyleneoxide, Independent, the alkyl which two or more components added or alkenyl ether carboxylate of propylene oxide and the butylene oxide, or an amide, alpha-sulfo fatty-acid salt or ester, an amino acid mold surfactant, alkyl, or alkenyl alkyl acid phosphate, Alkyl or the phosphoric ester system surfactant like alkenyl phosphate, It has the alkyl group or alkenyl radical of a sulfonic acid type amphoteric surface active agent, a betaine mold amphoteric surface active agent, a straight chain, or branched chain. Independent, the alkyl which two or more components added or the alkenyl ether of ethyleneoxide, propylene oxide, and the butylene oxide, or alcohol, It has the alkyl group or alkenyl radical of a straight chain or branched chain. Ethyleneoxide, Independent or polyoxyethylene alkyl phenyl ether which two or more components added of propylene oxide and the butylene oxide, A higher-fatty-acid alkanol amide or its alkylene oxide addition product, Sucrose fatty acid ester, fatty-acid glycerol monoester, alkyl, or alkenyl amine oxide, If it is the surfactant usually blended as a detergent constituent, such as a tetra-alkyl-ammonium-salt mold cationic surface active agent, all are usable, and it is desirable that they are sodium ion or potassium ion as a counter ion in the case of an anionic detergent. These surfactants are used as independent or two or more sorts of mixture.

[0027] As a builder and alkali chemicals, or an inorganic electrolyte, orthophosphate, A pyrophosphate, Tripoli acid chloride, a metaphosphate, a hexametaphosphoric acid salt, Phosphate, ethane -1, 1-diphosphonic acid, and its derivatives, such as a phytic acid salt, Ethane hydroxy - 1, 1, 2-triphosphonic acid, ethane -1, 2-dicarboxy - 1, 2-diphosphonic acid, Phosphonate, such as methane hydroxy phosphonic acid, 2-phosphono butane -1, 2-dicarboxylic acid, 1-phosphono butane - Phosphono carboxylate, such as 2, 3, 4-tricarboxylic acid, and alpha-methyl phosphono succinic acid, Amino acid salts, such as an aspartic acid and glutamic acid, a nitrilotriacetic acid salt, Amino poly acetate, such as an ethylenediaminetetraacetic acid salt and a diethylenetriamine pentaacetic acid salt, Polyacrylic acid, the Pori itaconic acid, a polymer lane acid, a maleic-anhydride copolymer, Polyelectrolytes, such as a carboxymethyl-cellulose salt, a polyethylene glycol, Non-dissociating giant molecules, such as polyvinyl alcohol, diglycolic acid, an oxydi succinic acid, Carboxymethyl malic acid, a gluconic acid, a citric acid, a lactic acid, a tartaric acid, Carboxymethyl ghosts, such as cane sugar and a lactose, the carboxymethyl ghost of pentaerythritol, The carboxymethyl ghost of a gluconic acid, benzene polycarboxylic acid, oxalic acid, Aluminosilicates, such as organic-acid salts, such as a malic acid, an oxydi succinic acid, and a gluconic acid, and a zeolite, Mineral salt, such as a carbonate, a sesquicarbonate, a sulfate, and a metasilicate, can be used as an alkali-metal salt. Moreover, inorganic compounds, such as organic substances, such as starch and a urea, and a sodium chloride, and a bentonite, can be used. Furthermore, triethanolamine, diethanolamine, monoethanolamine, tri-isopropanolamine, etc. can be used as organic alkali chemicals.

[0028] Although the detergent constituent of this invention contains the polyphenol oxidase of a surfactant and this invention etc. as a constituent like the above-mentioned, it can include the enzyme of others, such as anti-redeposition agents, such as bleaching agents, such as an amphoteric surface active agent, for example, perborate, and par carbonate, coloring matter, a builder, for example, a polyethylene glycol, polyvinyl alcohol, a polyvinyl pyrrolidone, and a carboxymethyl cellulose, a caking inhibitor, an antioxidant, for example, other oxidases and peroxidases, a protease, lipase, an amylase, and a cellulase, if needed if needed [the].

[0029] Although you may carry out with what kind of approach for blending an enzyme with the detergent constituent of this invention, blending by the shape of impalpable powder is not desirable for reasons of sanitation [of the detergent user by the raising dust at the time of detergent handling, or the operator in detergent industry / insurance], and it is desirable to carry out size enlargement to a solution condition or the configuration where dusting characteristics were pressed down beforehand. This size enlargement may be based on any of the approach of the Malmo grain usually used well, extrusion granulation, fluidized bed granulation, centrifugal fluidized bed granulation, or others, and especially the configuration of the enzyme blended with the detergent constituent of this invention is not limited to that

by which size enlargement was carried out by these approaches.
[0030] In a part of pulp process, inoculate the strain of this invention for the purpose of delignification and bleaching, and the polyphenol oxidase of this invention is made to produce, or the enzyme preparation of direct this invention is added, and the biotechnology PAL ping and biotechnology bleaching which are made to act on a chip, crushing pulp, etc. are mentioned to the useful application field.
[0031] As a natural product which has polyphenol in a part for the structured division, plant pigment and lignins, such as a flavonoid system, a xanthone system, and a melanin system, are known, and a polyphenol oxidase has the oxidation to these natural products. Moreover, AOX(s) from which toxicity has been a problem, such as dichlorophenol and trichlorophenol, are also made as for a polyphenol oxidase to a reaction substrate. So, also in the waste water treatment which contains these natural products and non-natural products, for example, the polyphenol oxidase of this invention is useful.
[0032] Moreover, the biosensor using the polyphenol oxidase of this invention can be used in order to act as the monitor of the aromatic compound in various water solutions which have pH of an acescence - weak alkaline region, and an organic solvent reflecting the property of this enzyme, and it is useful. Moreover, it is also possible to perform sterilization and inactivation of a microorganism or a virus efficiently in weak acidic - weak alkaline pH region using the reactivity of the phenoxyl radical generated by the polyphenol oxidase of this invention. That is, it is possible to give disinfectant [more powerful] by the phenoxyl radical generated in enzyme in addition to disinfectant [of the substrate of a polyphenol oxidase itself]. And when a sterilization processing object is contacted or taken in by the after body, or when being emitted into an environment, since the substrate of a polyphenol oxidase has changed to the matter with which toxicity was mitigated by oxidation, when required, it can attain the both sides of disinfectant and subsequent safety, and usefulness is high [the substrate].
[0033] Moreover, also in the polymer composition using the phenoxyl radical generated by the polyphenol oxidase, or quinones, the polyphenol oxidase of this invention is useful. Furthermore, since an autoxidation reaction and oxidation reaction of enzymatic catalyst-polyphenol can be advanced to coincidence when oxidizing the matter group containing easy-oxidizable polyphenol, such as catechols, use of the polyphenol oxidase of this invention is very effective because of efficient oxidation.

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EXAMPLE

[Example] A typical example is shown about this invention below, and it explains to it still more concretely. However, these are mere instantiation and this inventions are not these things restricted for seeing.
[0035] Example 1 : The flask of culture and rough purification, and 500ml ** of concentration is used for a culture apparatus. 0.134%Na2 HPO4 and 12H2 O, and 0.03%KH2 PO4, 1% maltose, 1% peptone, 0.1% yeast extract, 0.05%MgSO4 and 7H2 O, 0.1mM(s) CuSO4, 1mM MnCl2, 2mM CaCl2 It is 20% Na2 CO3 to the included 100ml culture medium. To what set pH to 7.8 in addition Bacillus licheniformis SD3003 (trust number FERM P-15383) was inoculated, culture temperature was lowered to 35 degrees C after 50 degrees C and the shaking culture of 16 hours, and culture for three more days was performed. The culture broth disinfected by 4-degree C centrifugal separation was obtained after culture. In order to refine and condense this further, ammonium sulfate fractionation was effective and was able to collect a great portion of polyphenol oxidase activity as precipitation in saturated-ammonium-sulfate concentration 20 to 60%. The obtained ammonium sulfate precipitation is 10mM(s). It dialyzed to the Bis-Tris-HCl buffer solution (pH7.0), in order to refine and condense further, ultrafiltration membrane was used, and the rough purification retentate solution (800 mU/ml) was obtained to the fractionation range of molecular weight 10,000-100,000.
[0036] Example 2: The substrate specificity of polyphenol compound oxidation reaction was investigated using the rough purification retentate solution of substrate specificity example 1 publication. In the room temperature (20 degrees C), it carried out by measuring the difference of the substrate of 0.05mM, and the enzyme addition in a 100 mMBis-Tris-HCl buffer solution (pH7.0) and an additive-free oxygen consumption coefficient. The result was shown in Table 1.
[0037]
[Table 1]

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4－アニシジン	+
o-フェニレンジアミン	+
フェルラ酸	+

[0038] Example 3: The molecular weight determination of molecular weight was performed using GFC (gel filtration chromatography). When analysis, and the aliquot and activity measurement of a rough purification retentate solution of example 1 publication were performed, elution of the polyphenol oxidase activity peak was carried out to the range of molecular weight 46,000-56,000 by 1.34%Na2 HPO4 and 12H2 O, 0.3%KH2 PO4, and HPLC using the GFC column (Shodex PROTEINKW-802.5, 2 ream) and UV detector (280nm) which equilibrated by rate-of-flow 1.0 ml/min by NaCl 1%. In addition, MW-Marker (HPLC) of Oriental Industry was used for molecular weight marker protein.
[0039] Culture with the 4:5l. cultivation tank of examples and concentration, 0.134% Na2 HPOof rough purification4 and 12H2 O, 0.03%KH2 PO4, 1% maltose, 1% peptone, 0.1% yeast extract, 0.05%MgSO4

and 7H2O, 0.1mM CuSO4, 1mM MnCl2 and 2mM CaCl2 from -- to the 5l. cultivation tank containing what added NaOH to the 3l. culture medium which changes 10%, and set pH to 7.8 Bacillus licheniformis SD3003 (trust number FERM P-15383) was inoculated, culture temperature was lowered to 35 degrees C after 50 degrees C and the shaking culture of 16 hours, and culture for three more days was performed. The culture broth disinfected by 4-degree C centrifugal separation was obtained after culture.

[0040] Next, a part of this culture broth was condensed as a with a molecular weight of 10,000 or more fraction by the mini tongue ultrafiltration system (Millipore Corp. make) which uses a mini tongue filter packet (CAT.NO.: PTGC0MP04, Millipore Corp. make). Furthermore, this concentration liquid is 200ppmNH(s)4 HCO3. It received, freeze drying was presented after dialysis, and the rough purification object was obtained as a freeze-drying article. The polyphenol oxidase activity of a freeze-drying article was 500 mU(s)/mg.

[0041] straight chain alkyl benzene sodium sulfonate (LAS) of 25 % of the weight of processings of the contamination cloth by the Example 5:enzyme content detergent, 5% of the weight of the polyoxyethylene lauryl ether, 15% of the weight of sodium tripolyphosphate, 6% of the weight of a sodium silicate, 1% of the weight of carboxymethylcellulose sodium, and 48% of the weight of Na2 SO4 from -- what added 0.1g of freeze-drying articles of example 4 publication in 10g of becoming index detergents was used as the enzyme combination detergent, and what has not added the freeze-drying article be used as enzyme the non-blending detergent. Moreover, the contamination cloth was prepared by adding 100 ppm Evans Blue (Wako Pure Chem Industry) 0.2ml in the center of cotton white cloth (5cmx5cm).

[0042] Next, after adding one contamination cloth and 10ml of water to 500ml ** beaker, washing processing was performed by 10mg adding and shaking an enzyme combination detergent, or enzyme a non-blending detergent for 12 minutes further. When the contamination cloth after processing was rinsed and air-dried, Y, y, and x values were measured with the color color difference meter (CR-200 and MINOLTA make) and Z value was further computed by the formula [Z=(1-x-y) Y/y], the enzyme combination detergent showed the improvement in a whiteness degree of 1.5 points compared with the enzyme non-blending detergent.

[Translation done.]

* NOTICES *

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] pH profile of the polyphenol oxidase of the SD3003 origin.
[Drawing 2] The temperature profile of the polyphenol oxidase of the SD3003 origin.
[Drawing 3] The graph which shows the temperature stability of the polyphenol oxidase of the SD3003 origin.
[Drawing 4] The graph which shows the pH stability of the polyphenol oxidase of the SD3003 origin.

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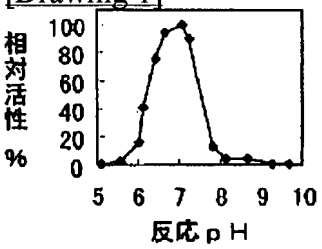
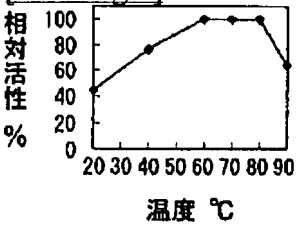
DRAWINGS

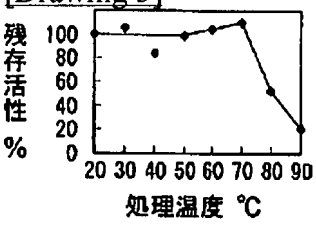
[Drawing 1]



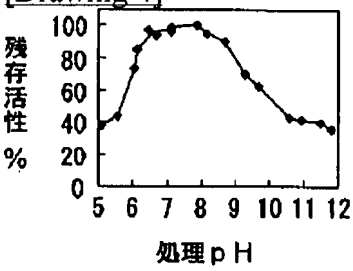
[Drawing 2]



[Drawing 3]



[Drawing 4]



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CLAIMS

[Claim(s)]
[Claim 1] The polyphenol oxidase of the bacteria origin.
[Claim 2] The polyphenol oxidase according to claim 1 which has the following property.
(1) Oxidize operation polyphenol.
(2) It has the optimum reaction pH in the optimum reaction pHpH7 neighborhood.
(3) It has optimum reaction temperature in optimum reaction temperature of 60-80 degrees C.
(4) The molecular weight measured by molecular weight GFC analysis is about 51,000.
[Claim 3] The polyphenol oxidase of the bacillus (Bacillus) group bacteria origin according to claim 1 or 2.
[Claim 4] The polyphenol oxidase according to claim 3 whose bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacillus licheniformis) or Bacillus natto (Bacillus natto).
[Claim 5] The polyphenol oxidase according to claim 1 or 2 which can be obtained from Bacillus licheniformis (Bacillus licheniformis) SD 3003 (trust number FERM P-15383).
[Claim 6] The art of the phenolic compound and alkoxyl group content aromatic series which are characterized by making the polyphenol oxidase of a publication act on any 1 term of claims 1-5, a halogenation phenolic compound, or an aromatic amine compound.
[Claim 7] The art of the coloring matter characterized by making the polyphenol oxidase of a publication act on any 1 term of claims 1-5.
[Claim 8] The art of the coloring wastewater characterized by making the polyphenol oxidase of a publication act on any 1 term of claims 1-5.
[Claim 9] The art of the microorganism or virus characterized by making the polyphenol oxidase of a publication act on any 1 term of claims 1-5.
[Claim 10] The art of the paper characterized by making the polyphenol oxidase of a publication act on any 1 term of claims 1-5, pulp, or fiber.
[Claim 11] Operation of a polyphenol oxidase given in any 1 term of claims 1-5 characterized by using with a cleaning agent, a detergent, or a surfactant.
[Claim 12] The detergent constituent characterized by including the polyphenol oxidase of a publication in any 1 term of claims 1-5.
[Claim 13] Operation of a polyphenol oxidase given in any 1 term of claims 1-5 characterized by using with the matter which has a peroxidase operation.
[Claim 14] Operation of a polyphenol oxidase given in any 1 term of claims 1-5 characterized by being independent, or combining two or more air, oxygen, ozone, a hydrogen peroxide, hydrogen-peroxide precursors, peroxy-acid precursors, or peroxy acids, and using them as an oxidizer.
[Claim 15] Operation of a polyphenol oxidase given in any 1 term of claims 1-5 characterized by using with an oxidase and its substrate.
[Claim 16] The manufacture approach of a polyphenol oxidase given in any 1 term of claims 1-5 characterized by cultivating bacillus (Bacillus) group bacteria.
[Claim 17] The manufacture approach of a polyphenol oxidase according to claim 16 that bacillus

(Bacillus) group bacteria are Bacillus licheniformis (Bacillus licheniformis) or Bacillus natto (Bacillus natto).
[Claim 18] The manufacture approach of a polyphenol oxidase according to claim 16 that bacillus (Bacillus) group bacteria are Bacillus licheniformis (Bacillus licheniformis) SD 3003 (trust number FERM P-15383) or its variant.
[Claim 19] The bacillus (Bacillus) group bacteria which produce the polyphenol oxidase of a publication in any 1 term of claims 1-5.
[Claim 20] Bacillus licheniformis SD 3003 (trust number FERM P-15383) (Bacillus licheniformis).

[Translation done.]